Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at http://about.jstor.org/participate-jstor/individuals/early-journal-content.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

AMERICAN
JOURNAL OF BOTANY

VOL. VI NOVEMBER, 1919 No. 9

A STUDY OF SOME FACTORS IN THE CHEMICAL STIMULATION OF THE GROWTH OF ASPERGILLUS NIGER

ROBERT AARON STEINBERG

(Concluded from the October Journal)

The results obtained in these attempts to effect a still further increase in the yield by the addition of free acid during growth as compared to its addition before inoculation are not conclusive. While experiments 28 and 29 would lead one to suspect that the optimum hydrogen-ion concentration for growth varies during the growth of the organism, experiments 30 and 31 do not seem to bear out this assumption. On the other hand, it must be admitted that the procedure followed in experiments 30 and 31 is not wholly comparable to that in experiments 28 and 29 with respect to the degree of the variations in acidity, imposed as they are in one instead of several days. I believe, therefore, that the results of experiments 28 and 29 should be given greater weight than those of experiments 30 and 31.

The contention of Nikitinsky (48) that *A. niger* eliminates a "stimulative" substance into the nutrient solution whose action is frequently inhibited by excessive increase in acidity of the cultures seems to me unproven. A glance at experiment 2 (of Nikitinsky) shows that the acidified cultures produce a greater yield (first harvest). Reference, moreover, to experiments 6, 7, 8, 9, and 11 indicates that in the majority of cases (see, however, *Penicillium griseum* grown on NH_4NO_3, experiments 7 and 11) when the acid formed in the first culture has been neutralized, the resulting yield in the second culture does not exceed that in the first. On the other hand, the yields for the second cultures, without neutralization of the acid formed by the first, show as a rule an acceleration of growth. The growth accelerations usually obtained through addition of excess $CaCO_3$, while without doubt in some cases due to a reduction in excessive acidity (experiment 23, culture 3) are in the majority of cases due to other causes, since previous observers (Wehmer, 68; Butkewitsch, 8) agree that a decrease in growth always occurs.

We may turn now to some results obtained by the use of a method of purification of the culture medium. The marked increase in yield following the addition of increased amounts of magnesium sulphate suggested the question whether the apparent action of the magnesium sulphate is due to

[The Journal for October (8: 311–356) was issued Nov. 20, 1919.]

the salt itself or to the impurities present in the salt. Recourse was had to further chemical purification.

The method of purification used depends on the fact that the "stimulants" in question are salts of cations whose hydroxides (and carbonates also) are quite insoluble and but weakly ionized. The barium carbonate method (66) of separation of ferric, aluminum, chromic, titanic, and uranyl salts from manganese, nickel, cobalt, zinc, and ferrous salts has long been known and is a standard method in the chemical laboratory. In using this method of separation it is necessary to carry out the hydrolysis in the cold, since on warming the salts of the bivalent metals are also hydrolyzed to an appreciable extent and are then precipitated by the barium carbonate.

It is evident, however, that the use of barium carbonate would be objectionable from a physiological point of view, since some barium necessarily goes into solution. Calcium carbonate, however, with use of higher temperatures (20 minutes at 14½ pounds in the autoclave) apparently causes the precipitation of these metals (iron, zinc, etc.) as effectually, and was therefore used in place of the barium carbonate.

The details of the method of treating the Pfeffer solution as I have used it are as follows: The Pfeffer solution is prepared by weighing out the sugar and salts (except iron sulphate) into the same beaker, redistilled water is added, and the compounds are permitted to go into solution. The solution is now placed in liter pyrex flasks, 15 grams of dry $CaCO_3$ (Baker's "Analyzed") are added per liter, and the neck of the flask is plugged with absorbent cotton. The contents are now thoroughly rotated so as to insure mixing and placed in the autoclave for twenty minutes at 14½ pounds pressure. On removal from the autoclave the flask is again rotated to cause mixing of the contents and set aside in a cupboard for 12 to 24 hours, or even for several days. When required for use it is necessary only to remove the cotton plug and to decant the supernatant liquid into a beaker. Fifty cc. of culture medium are now poured into each flask, the flask is plugged with absorbent cotton and sterilized. The substance to be tested, iron, zinc, etc., is added the next morning shortly before inoculation.

The Pfeffer solution so treated is still slightly acid to litmus, gives excellent tests for phosphates and sulphates, and without doubt contains calcium. The $CaCO_3$ residue contains appreciable amounts of phosphates and sulphates from the nutrient solution, but a test for sugar (Fehling solution) gave negative results. A slight precipitate forms in the treated solution on sterilization. The composition of this precipitate as well as that of the nutrient medium has as yet not been definitely determined, but it is planned, in connection with the testing of various other heavy metals and their combinations as substitutes for iron and zinc, to ascertain analytically just how and in what degree the components of the Pfeffer nutrient solution undergo modification as the result of the treatment. Reference to the earlier experiments given in this paper indicate that the variations in the proportions and concentrations of the Pfeffer nutrient solution which probably occur are not factors of primary significance.

With a culture medium so treated I have obtained on addition of both iron and zinc a dry weight of 1.158 g., and with the untreated Pfeffer solution a yield of 1.024 g. Both these values are maximum values for the W strain when supplied with iron and zinc. The maximum yield I have at any time obtained is 1.174 g. in culture with my original strain grown on Pfeffer solution and in Jena glass.

32. The effect of treatment of the magnesium sulphate as shown by its action on growth.

Strain W, 7			
(*A*) 10 : 5 : 130		(*B*) 10 : 5 : 130 ($MgSO_4 \cdot 7H_2O$ Purified).	
No Zinc	1.0 Mg. Zn/L	No Zinc	1.0 Mg. Zn/L
0.718 g.	0.757 g.	0.431 g.	0.785 g.
0.776	0.768	0.432	0.849
0.772	0.752	0.456	0.839
0.668	0.836	0.472	0.839
0.725	0.882	0.491	0.923
0.732	0.799	0.456	0.847

Solution (*B*) was prepared with $MgSO_4.7H_2O$ that had been autoclaved in 26% solution with 30 g. $CaCO_3$ per liter, the solution after treatment being decanted and used in making up the Pfeffer solution. The "no zinc" under solution (*B*) refers to the fact that none was added to the magnesium sulphate before its treatment; "+ 1.0 mg. Zn/L" refers to the fact that enough zinc was added to the magnesium sulphate before treatment so that in the preparation of the Pfeffer solution this concentration of zinc should result. That is, the 26 percent solution of $MgSO_4.7H_2O$ contained in the latter instance 2.0 mg. Zn/L before treatment with $CaCO_3$. The zinc cultures were sterile; excess zinc. p of ll cultures on harvest was 1–2.

The results of experiment 32 indicate clearly that the treatment with $CaCO_3$ acts essentially through a reduction of the zinc content of the $MgSO_4.7H_2O$. With solution (*A*), zinc is present in too high concentration; in solution (*B*), the treatment has apparently removed an appreciable amount of zinc, as is evidenced by the increased yield and increased fructification. As respects solutions (*A*) and (*B*) in those cases in which no zinc was added, we note that the treatment results in a reduction in yield. On the other hand, a comparison of the cultures in solution (*B*) with and without zinc would indicate that the action of the $CaCO_3$ cannot consist in any action on the $MgSO_4.7H_2O$, but must depend on the removal of the impurities present.

After decantation of the solution from the $CaCO_3$, the residue (15 g. $CaCO_3$ + 100 cc. Pfeffer solution) was used as a medium. An excellent "stimulated" culture resulted, a demonstration that the "stimulative" substances (zinc) had been removed from the Pfeffer solution by the $CaCO_3$. The cultures in the treated solution exhibited much scantier growth and spore formation than the control. The conidiophores in the former had apparently been reduced to a minimum length.

33. On the removal of added zinc from the culture medium by treatment with $CaCO_3$.

Strain W, 7			
Control	1.0 Mg. Zn/L + 30 g. $CaCO_3$/L		
	$CaCO_3$ Added Before Sterilization		$CaCo_3$ Added After Sterilization
	$CaCO_3$ Not Removed	Decanted Solution	
0.078 g.	0.545 g.	0.039 g.	0.759 g.
0.082	0.581	0.050	0.993
0.091	0.802	0.042	0.750
0.087	0.780	0.043	0.476
0.090	0.558	0.039	0.952
0.086	0.653	0.043	0.786

Cultures prepared from solution decanted from $CaCO_3$	
HNO_3	
0.1 cc./flask	0.084 g.
0.1	0.103
0.2	No growth
0.2	" "

As can be seen from experiment 33, the evidence for the removal of zinc by this method of treatment with $CaCO_3$ is quite conclusive. Furthermore, it is to be noted that the addition of $CaCO_3$ to a zinc culture (1 mg. Zn/L) depresses the yield least when added after sterilization, to a greater extent when added before sterilization, and most when it is added before sterilization and the supernatant liquid is removed by decantation. That is, the presence of calcium carbonate in the cultures causes a decrease in the rate of growth similar to that described by Wehmer (68), Butkewitsch (8), and Nikitinsky (48). If the calcium carbonate is added before sterilization, the effect of the heating in the sterilization process will be to cause the hydrolysis and precipitation of the zinc. The constituents of this residue go slowly into solution, thus affording zinc and iron necessary for the increased growth. If, however, the residue of calcium carbonate with its contained zinc, etc., is removed, as is the case when the solution is decanted, then growth is sharply limited. Indeed, in the latter case it is less than in the untreated Pfeffer solution.

I next tested whether the addition of nitric acid to the treated solution influences the yield. Two duplicates with 0.1 and 0.2 cc. HNO_3 per flask respectively were inoculated. In the latter no growth occurred, as was the case with the Pfeffer solution without treatment (experiments 20 and 23). With 0.1 cc. HNO_3 per flask, the growth was slightly accelerated, and, as was found to be the case with the untreated Pfeffer solution, the hyphae were opaque and the membranes wrinkled. Spore formation had been partially suppressed under these conditions. The small increase in growth on increasing the acidity indicates that the decreased acidity as a result of treatment cannot account entirely for the results observed.

With respect, therefore, to the marked acceleration of growth observed with very high concentrations of magnesium sulphate, I am of the opinion that the results observed are due to the presence of minute amounts of substances (probably zinc, iron, etc.) as impurities in the magnesium sulphate.

As I have already had occasion to state in a former publication (65) "it is perhaps questionable whether thus far anyone has grown *A. niger* in the complete absence of zinc." Accidental factors are exceedingly difficult to control, *e.g.*, variation in purity of a compound—even of different samples from the same bottle; contamination with dust, etc., from the air and from the cotton plugs of the flasks, etc.

The use of the hydrolytic purification method is well adapted for the study of the influence of zinc and iron on the growth of *A. niger*. Furthermore, by the use of this method I have been able to obtain a culture medium with which the non-addition of either iron or zinc markedly decreases the acceleration of growth by the other. This relation between iron and zinc is brought out in experiments 34 to 37 inclusive.

34. The effect of the addition of iron and zinc to the treated solution.
Each value for one culture.

Strain W, 11

	Treated Pfeffer Solution	
Composition of Solution	Yield	P_H
Control	0.018 g.	4
Control	0.013	4
+ 1 mg. $Fe_2(SO_4)_3$/flask	0.044	2–3
5 "	0.026	4
20 "	0.099	2–3
+ 1 mg. Zn/L	0.040	4
10 "	0.045	4
50 "	0.056	4
+ 1 mg. $Fe_2(SO_4)_3$/flask + 1 mg. Zn/L	0.731	1–2
1 " " " + 10 mg. Zn/L	0.787	1–2

The evidence shows that neither ferric sulphate even in 0.4 percent concentration nor zinc in a concentration of even 50 mg. Zn/L added *alone* can cause any but very slight increases in growth. If we add, however, but 0.002 percent $Fe_2(SO_4)_3$ and 1 mg. Zn/L *together*, we obtain the characteristic phenomenon of "stimulation." The high yields obtained by the combined action of zinc and iron have not been demonstrable by any other method of experimentation, neither has the markedly limited growth in the absence of zinc or of iron from the culture medium. I have further tested the possibility that the results with the treated solution are due to the calcium ion and the much lower acidity. The influence of the latter factor has been already emphasized. In experiment 35 I have attempted to duplicate the conditions with respect to acidity and the presence of calcium ion in the treated solution by the addition of ammonium hydroxide and $Ca(NO_3)_2$ to the untreated Pfeffer solution. The amount of $Ca(NO_3)_2$ is of course excessive in this solution as compared with the treated nutrient solution where the entire amount of $CaCO_3$ added amounted to only one and one half percent. The use of one percent $Ca(NO_3)_2$ was due to an error in mixing, it being estimated that a liter of solution had been prepared whereas in reality it was only a half liter.

35. The effect of increased acidity of the treated solution together with that of the addition of iron and zinc.

Strain W, 12

Yield	Control	Sporulation	H_3PO_4/Flask			Sporulation
	p_H		Yield		p_H	
0.012 g.	4	Fair	0.2 cc.	0.097 g.	2–3	Excellent
0.011	4	Fair	0.3	0.125	2–3	"
0.011	4	Fair	0.4	0.121	2–3	Fair
			0.5	0.041	2–3	Sterile

$Fe_2(SO_4)_3$/Flask

$Fe_2(SO_4)_3$	Yield	p_H	Sporulation
1 mg.	0.030 g.	3–4	Fair
5	0.033	3–4	"
20	0.326	2–3	Excellent
50	0.655	1–2	Sterile
100	0.823	1–2	"
200	0.864	1–2	"

Mg. Zn/L

Mg. Zn/L	Yield	p_H	Sporulation
1	0.115 g.	3–4	Fair
10	0.067	3–4	"
50	0.054	3–4	"
100	0.100	2–3	"
150	0.051	3–4	Practically sterile
200	0.055	3–4	" "

1 Mg. $Fe_2(SO_4)_3$/Flask

Mg. Zn/L	Yield	p_H	Sporulation
0.05	0.288 g.	2–3	Excellent
0.2	0.562	1–2	Good
0.5	0.752	1–2	Good
1.0	0.710	1–2	Sterile

0.1 Mg. Zn/L

$Fe_2(SO_4)_3$/Flask	Yield	p_H	Sporulation
0.5 mg.	0.473 g.	2–3	Excellent
1.0	0.727	1–2	Good
5.0	0.685	2–3	"

1 Mg. $Fe_2(SO_4)_3$/Flask

H_3PO_4/Flask	Yield	p_H	Sporulation
0.2 cc.	0.134 g.	2–3	Excellent
0.2	0.128	2–3	Excellent
0.4	0.110	2–3	Sterile

0.1 Mg. Zn/L

H_3PO_4/Flask	Yield	p_H	Sporulation
0.2 cc.	0.594 g.	1–2	Sterile
0.3	0.552	1–2	Sterile
0.4	0.337	1–2	Sterile

0.2 Cc. H_3PO_4/Flask

$Fe_2(SO_4)_3$/Flask	Yield	p_H	Sporulation
0.5 mg.	0.122 g.	2–3	Excellent
1.0	0.090	2–3	"
5.0	0.172	2–3	"
20.0	0.845	2–3	Sterile

0.2 Cc. H_3PO_4/Flask

Mg. Zn/L	Yield	p_H	Sporulation
0.075	0.654 g.	1–2	Sterile
0.2	0.477	1–2	"
0.5	0.456	1–2	"

Pfeffer Solution

Control

Mg. Zn/L	Yield	p_H	Sporulation
0	0.253 g.	3–4	Excellent
1	0.895	1–2	Good
10	0.909	1–2	"
25	0.972	1–2	"

+ 1% $Ca(NO_3)_2$ + 0.1 Cc. NH_4OH/Flask

Mg. Zn/L	Yield	p_H	Sporulation
0	0.398 g.	3–4	Excellent
1	0.850	1–2	Good
10	0.887	1–2	"
25	0.787	1–2	"

The $Ca(NO_3)_2$ used was the Baker's "Analysed." In both experiments 34 and 35 as well as in experiment 36 only 15 g. $CaCO_3$ per liter were used in effecting the purification.

Here again it is shown that the addition of both zinc and iron is necessary in order to obtain marked increases when the treated Pfeffer solution is used. Either alone in much higher concentration is apparently ineffective, growth being limited by the absence of the other. Nevertheless, with very high iron concentrations a marked acceleration of growth occurs. The same is also true for iron in acidified culture (0.2 cc. H_3PO_4 and 20 mg. $Fe_2(SO_4)_3$ per flask) as well as for zinc only. It should be emphasized that both the iron and the zinc salt used in these experiments are of a purity sufficient for analytical work but hardly come up to the required standard as respects physiological work of this character. Enough iron is still probably present in the treated solution together with that added with the zinc salt and in the spores to enable a limited (0.654 g.), but still marked, increase in growth on addition of zinc to take place. Asō (1) has reported that in the case of *Aspergillus Oryzae* the spores contain appreciable amounts of Fe_2O_3 (0.14 percent). With a similar iron content in the spores of *A. niger* it is quite evident that enough iron might be added to influence the results in view of the heavy inoculations necessary in these experiments for uniform results.[2] The increased acidity, since its action is to aid that of the zinc, makes its effect evident in the concentrations employed. This effect of increased acidity of the nutrient solution has already been noted in connection with the increased effectiveness of zinc in the untreated Pfeffer solution as well as in connection with the increased effectiveness both of iron and of zinc in the treated Pfeffer solution. This relation between acidity and the action of heavy metal appears to be of general significance, since, as already noted, it seems to hold also for the higher plants.

Inspection of the values obtained in the presence of calcium ion, in the Pfeffer nutrient solution of about the same acidity as the treated solution, shows that the influence of these two factors on the results obtained in the treated Pfeffer solution can be disregarded. Even in the presence of much more calcium than is present in the treated solution as well as of a decreased acidity corresponding approximately to the treated solution (slightly acid to litmus), the customary accelerations of growth with small amounts of zinc are obtained.

The next experiment (experiment 36) was planned to test the value to be placed on the accelerations of growth obtained in the presence of iron and zinc salt alone. In this experiment two Kahlbaum "Zur anal." compounds [$FePO_4$ and $Fe_3(PO_4)_2$] were used, the phosphates being selected for the very simple reason that neither the nitrates nor sulphates in a sufficient degree of purity were available. The relative insolubility of these salts is hardly a factor, since the addition of iron in soluble form results in the precipitation of the phosphates in the nutrient solutions.[3]

The spores were brown in many of the zinc-plus-iron cultures. Accompanying the brown spores, many papilla-like protuberances on the upper surfaces of the membranes were visible. These papillae have never been observed in zinc-free cultures, and in zinc cultures containing sufficient iron have been observed only at irregular intervals. Both the iron salts are the Kahlbaum "Zur. anal."; the zinc salt, the Baker's "Analysed."

---

[2] Approximately 4 mg. dry weight of spores, etc., per flask.

[3] I have estimated that the amount of material (dry-weight) added at the time of inoculation is on the average approximately 3–4 mg. to each flask. The actual amount of course probably differed widely from flask to flask.

We note that the growth with either zinc or iron salts alone is sharply limited even with concentrations that may physiologically be termed very high. The presence of free acid in the cultures to which the iron salts are added, while it does cause accelerations of growth, can not in view of their limited magnitude be held as the factor responsible for the results with the treated solution. Again we note also that there can be no question that sufficient sugar is present in this solution to form a yield as great as that I obtained with the untreated Pfeffer solution (1.024 g.). The treated Pfeffer solution contains all the components that *A. niger* requires for growth with the exception of sufficient iron and zinc.

36. The effect of increased acidity of the treated solution together with that of the addition of iron and of zinc.

Strain W, 12. Treated Solution

$FePO_4$/Flask

$FePO_4$/Flask	Yield	P_H	Sporulation
0 mg.	0.004 g.	3–4	Fair
1	0.016	3–4	"
5	0.004	3–4	"
20	0.010	3–4	"
50	0.021	3–4	"
100	0.030	3–4	"
200	0.065	3–4	"

$Fe_3(PO_4)_2$/Flask

$Fe_3(PO_4)_2$/Flask	Yield	P_H	Sporulation
0 mg.	0.010 g.	3–4	Fair
1	0.032	3–4	"
5	0.045	3–4	"
20	0.094	2–3	Excellent
50	0.111	2–3	Good
100	0.147	3–4	"
200	0.213	3–4	"

0.2 Cc. H_3PO_4/Flask

$FePO_4$/Flask	Yield	P_H	Sporulation
0 mg.	0.117 g.	2–3	Excellent
1	0.123	2–3	"
5	0.157	2–3	"
20	0.154	2–3	"
50	0.187	2–3	"
100	0.277	2–3	"
200	0.273	1–2	"

$Fe_3(PO_4)_2$/Flask	Yield	P_H	Sporulation
0 mg.	0.071 g.	2–3	Excellent
1	0.106	2–3	"
5	0.176	2–3	"
20	0.170	2–3	"
50	0.254	2–3	"
100	0.437	2–3	"
200	0.580	2–3	"

0.1 Mg. Zn/L

$FePO_4$/Flask	Yield	p_H	Sporulation
0 mg.	0.076 g.	3–4	Fair
1	0.660	1–2	Excellent
5	0.822	1–2	Good
20	0.772	1–2	Good
50	0.840	1–2	Excellent
100	0.664	1–2	"
200	0.591	1–2	"

$Fe_3(PO_4)_2$/Flask	Yield	p_H	Sporulation
0 mg.	0.070 g.	3–4	Fair
1	0.553	2–3	Excellent
5	0.665	1–2	"
20	0.784	1–2	"
50	1.006	1–2	"
100	1.158	1–2	"
200	1.012	1–2	"

Mg. Zn/L

Mg. Zn/L	Yield	p_H	Sporulation
0	0.005 g.	3–4	Fair
0.025	0.047	3–4	"
1	0.079	3–4	"
5	0.037	3–4	"
10	0.030	3–4	Practically sterile
25	0.040	3–4	Practically sterile
50	0.037	3–4	Practically sterile
100	0.072	3–4	" "
150	0.040	3–4	" "
200	0.062	3–4	" "

My experiments with the treated Pfeffer nutrient solution indicate that iron and zinc are in a different class as to efficiency from any of the other "stimulative" elements (cobalt, nickel, etc.) so far tested. It seems probable from these experiments, further, that the large growths obtained apparently with the use of zinc alone in my earlier experiments (64, 65) were in reality due to the combined action of zinc and iron, the iron being introduced as an impurity with the sugar or the other salts together with that intentionally added (45). The very limited growth obtained in the treated solution when either is used alone indicates that only by their combined action could the very marked increase of growth obtained in the experiments referred to have been induced. A further study of the composition of the treated solution is necessary, but there can be no question that it still contains as noted the necessary salts and sugar. Experiment 35, and experiment 33 as well, show that neither the calcium ion nor any compounds which it may form has any particular effect on the growth. The evidence seems conclusive that the treatment really results in a more thorough removal from the medium of all salts of zinc and iron than had hitherto been achieved in such experiments on growth "stimulation." While the prolonged exposure to high temperatures (about 150° C.) of such a mixture as the Pfeffer solution with its sugar and salts and the $CaCO_3$ may lead to fundamental rearrangements, the chemistry of the process as used for the removal of iron and zinc is well understood and is utilized as a standard procedure for the separation of the trivalent from the bivalent metals of the ammonium sulphide group.

In the light of these experiments, I am therefore inclined to the opinion that the increased growth of *A. niger* which I have obtained in Pfeffer solution on addition of zinc salt, and which other workers (see review of the literature) have also obtained in this manner, is due not alone to the zinc added but to the fact that iron is present, in the same manner that the increased growth obtained in the presence of an iron salt is due not alone to the iron added but to the fact that zinc is present.

Lipman and Gericke (34) in a carefully made series of experiments find that when a sodium salt in toxic amount is added to the soil in pot experiments the injurious effects can be eliminated by the addition of copper and zinc salts. The thirteen tables of data submitted indicate that, as compared with the controls with alkali salt in toxic concentration, the presence of copper or zinc salt results as a rule in a greater dry weight of straw, grain, and roots. The importance of the practical application of these results as a means for the utilization of "alkali" soils is obvious.

The marked increases in yield obtained by these authors previously (33), under the same conditions except that no alkali salts were added, would indicate the possibility that in these as in their present investigations the acceleration in growth may be due to the action of copper, zinc, etc., primarily as "stimulants." That is, an acceleration in growth occurs in the presence of both non-toxic and toxic concentrations of "alkali" salts when zinc, copper, etc., are added. The effects of $CuCO_3$ in the presence of toxic concentrations of Na_2CO_3 is, as compared to that obtained with $NaCl$ or Na_2SO_4, not marked according to the authors. In cultures of *Aspergillus niger* the effect of the addition of substances causing a decrease in acidity is, as shown in experiments 23, 25, 26, etc., markedly to decrease the "stimulative" action of zinc. Zinc salts and acidity have a mutual effect on growth, reproduction, etc., in cultures of *A. niger*. Acidity within certain limits markedly favors the growth of *A. niger*. Acidity of the soil is supposed to be detrimental to cereal crops, but here again probably only when it exceeds certain limits. If, as I believe to be the case, the facts determined for the nutrient solution with *Aspergillus niger* hold also in general for the higher plants, then maximum growth will be best assured by a relatively (compared to the optimum of the particular plant) lower acidity and the presence of heavy metals (iron, zinc, manganese, etc.) in predetermined amount. The practical application of these facts to diminish the action of heavy metal salts in toxic concentrations is well shown, even if unconsciously, by the practise of applying a good dressing of lime as an effective antidote (60, p. 51) whenever under special conditions infertility is traced to any of these metallic salts. The decreased acidity, perhaps also the partial precipitation of the heavy metals present in the soil solution, leads to increased growth both by decreasing their concentrations and by a marked diminution of the action of the heavy metals resulting from the increased alkalinity.

My observations on the acceleration of growth by increased acidity, etc., are of interest in connection with MacDougal and Spoehr's (36) studies on the correlation between growth and the imbibition of water by colloids as influenced by variation in the acidity. Growth in Opuntia, they conclude, is decreased by the excessive accumulation of acids in darkness. The decreased acidity of the tissues due to the action of light during the forenoon is accompanied by an acceleration of growth. Furthermore, the imbibition by protein and carbohydrate colloids, and by mixtures of the two in varying proportions, differs as to acid, water, and alkali. The imbibition phenomena of gelatine, they find, parallel more closely the growth phenomena of the animal organism; whereas those of agar parallel more nearly the growth phenomena of plants. One of their colloidal mixtures compounded—gelatine 100 + agar 1—reacts as regards imbibition in the presence of acid, of water, and of alkali in a manner greatly similar to the growth rates of *A. niger* under similar conditions of variation in the reaction of the medium. Thin plates of this mixture, they find, increase in thickness to 520 percent of the original thickness in *n*/100 NaOH, to 750 percent in water, and to 1100 percent in *n*/100 HCl.

It is always to be remembered, however, that in *A. niger* we have increased assimilation of food materials as shown by the increased dry weight.

The phenomenon of imbibition of water by colloids has also been used by Fischer (15) in the interpretation of oedematous swellings in the animal organism. The imbibition of water by colloids like gelatine, gluten, etc., in the presence of various compounds and degrees of acidity is paralleled, he shows, to a high degree by the behavior of organized animal tissues under the same conditions. Whereas, however, the use by Fischer of certain acidities led presumably only to an increased water content, so that the total dry weight is not increased, in *A. niger*, as just noted, under analogous conditions an increase in the dry weight results. That perhaps increase in dry weight occurs to some extent through hydration of the colloids, as has been found by Pauli to take place with gelatine, is not, however, improbable. Still, while there may be some question whether mere hydration can be referred to as growth, there can be no doubt that we have acceleration of true growth in *A. niger*.

The growth accelerations of *A. niger* resulting from "chemical stimulation" are hardly to be attributed in their entirety to, nor apparently are they paralleled by, the imbibitional capacity of the fungus for water. Growth as we are studying it involves *cell multiplication* and *increase in weight* of organic compounds. The root-tip has long served as the classical object for the illustration of the different kinds of growth: cell division, increase in dry weight, and increase in volume; not to mention differentiation. At the apex the cell divides, increases in volume here being due to increases in the organic compounds of the cell, and hence in dry weight due to the imbibition and assimilation of foods and water. Farther back, in what is commonly referred to as the zone of elongation, the rapid increase in size of the cells appears in the main to be due to absorption of water and its accumulation in vacuoles, without necessarily an increase in the dry weight of protoplasm present. Lastly we note that certain cells undergo a still further modification of form in the zone of differentiation. The distinction between the different kinds of growth is not always sharply marked. Neither are the primary forces making for the intake of water and foods by the cell determined as yet with any certainty. An increase in protoplasmic volume may be attributed to an increased imbibition of water, just as an increase in size of the vacuoles is attributed to osmotic phenomena. The absorption of foods by the organism results in the formation of colloids, which, however, would not necessarily increase the imbibitional capacity of the protoplasm. Those materials which accumulate as soluble bodies in the vacuoles and lead to increase in size of course play a very important part in the elongation of the so-called growing region of the root. In *A. niger* the thickening of the cell walls (differentiation) also probably contributes largely to the end results of the growth acceleration in the presence of zinc and iron. It is certainly of interest that in animal tissues, in tissues of cacti, and in the hyphae of *A. niger*, increase in acidity within certain limits (*i.e.*, optimum p_H or acidity) may lead to increased absorption of water, or of nutrients that are further elaborated, or of both.

It is clear that increased acidity and the specific effect of certain heavy metals are not the sole means by which growth is accelerated and spore formation retarded or suppressed in the case of "chemical stimulation" of *A. niger*. Such compounds as chloroform, ether, etc., which act as "stimulants," do not, as far as we at present know, increase the acidity of the nutrient solution. No purpose is served by speculations attempting to suggest uniformity where none may exist.

My studies were made under the direction of Professors W. G. Marquette and R. A. Harper. To both I am greatly indebted for advice, aid, and encouragement.

SUMMARY

1. Increased acidity of the Pfeffer nutrient solution within a certain range results in the exhibition in *Aspergillus niger* cultures of growth "stimulation" like, but less in amount than, that obtained by addition of salts of certain heavy metals. The appearance of the cultures duplicates exactly, in decrease in sporulation and in formation of opaque hyphae resulting in thick, white, wrinkled membranes, that of the classic zinc cultures.

2. Increase in acidity of the nutrient solution supplements the action of heavy metals (zinc, iron); decrease in acidity detracts from the action of heavy metals.

3. Salts of the heavy metals which act as "stimulants" hydrolyze as a rule in aqueous solution so as to result in an increased acidity, and this increase in acidity, though slight, owing to the low concentration in which the salts are used, may possibly be considered as rendered effective through membrane concentration. Sodium silicate is an exception to this rule of increase in acidity by "stimulants," and it was found in my experiments not to produce effects like those of zinc on the growth, sporulation, etc., of *A. niger*.

4. The progressive increase in acidity of the culture consequent upon the addition of a "stimulant" is, at least in major part, a result of the activities of the organism, and may in turn be concerned also in the acceleration of growth and in the retardation of spore formation.

5. Autoclaving at 14½ pounds the Pfeffer nutrient solution with calcium carbonate leads apparently to the removal to a high degree of the remaining traces of iron and zinc, and probably of all heavy metals present.

6. Evidence is given that in the Pfeffer nutrient solution thus treated, practically no growth takes place; that the addition of either iron or zinc alone causes only a very slight increase in growth, but that when zinc and iron are added together a marked increase in growth takes place.

LIST OF PAPERS REFERRED TO IN THE TEXT.

1. **Asō, K.** The chemical composition of the spores of *Aspergillus Oryzae*. Bull. Coll. Agr. Tokyo Imp. Univ. **4**: 81. 1900.
2. **Ayers, S. H.** Hydrogen ion concentrations in cultures of streptococci. Journ. Bact. **1**: 84. 1916.
3. **Benecke, W.** Die zur Ernährung der Schimmelpilze nothwendige Metalle. Jahrb. Wiss. Bot. **28**: 493. 1895.
4. **Bertrand, G., and Javillier, M.** Influence du manganèse sur le développement de l'*Aspergillus niger*. Compt. Rend. **152**: 225. 1911.
5. ——— — ———. Influence combinée du zinc et du manganèse sur le développement de l'*Aspergillus niger*. Compt. Rend. **152**: 901. 1911.
6. **Braconnot, H.** See Czapek, F. Biochemie der Pflanzen, 2te Aufl. **1**: 148.
7. **Brenchley, W. E.** Inorganic plant poisons and stimulants. Cambridge, 1914.
8. **Butkewitsch, W.** Unwandlung der Eiweissstoffe durch die niederen Pilze im Zusammenhange mit einigen Bedingungen ihrer Entwickelung. Jahrb. Wiss. Bot. **38**: 147. 1902.
9. **Clark, J. F.** Electrolytic dissociation and toxic effect. Journ. Phys. Chem. **3**: 263. 1899.
10. **Clark, W. M., and Lubs, H. A.** The colorimetric determination of hydrogen ion concentration and its application in bacteriology. Journ. Bact. **2**: 191. 1917.
11. **Currie, J. N.** The citric acid fermentation of *Aspergillus niger*. Journ. Biol. Chem. **31**: 15. 1917.
12. **Czapek, F.** Biochemie der Pflanzen I. 2te Aufl. Jena, 1913.
13. **Denham, H. G.** The electrometric determination of the hydrolysis of salts. Journ. Chem. Soc. **93**: 41. 1908.

14. **Erdmann, H.** Lehrbuch der anorganischen Chemie. 3te Aufl. 1906.

15. **Fischer, M. H.** Oedema. 1910.

16. **Fred, E. B.** Ueber die Beschleunigung der Lebenstätigkeit höherer und niederer Pflanzen durch kleine Giftmengen. Centralbl. Bakt. **31**: 185. 1912.

17. ——**, and Loomis, N. E.** Influence of H-ion concentration of medium on the reproduction of alfalfa bacteria. Journ. Bact. **2**: 629. 1917.

18. **Gile, P. L., & Carrero, J. O.** Assimilation of iron by rice from certain nutrient solutions. Journ. Agr. Res. **7**: 503. 1916.

19. **Hartwell, B. L., and Pember, F. R.** The presence of aluminium as a reason for the effect of so-called acid soil on barley and rye. Soil Science **6**: 259. 1918.

20. **Hoagland, D. R.** The effects of hydrogen and hydroxyl ion concentration on the growth of barley seedlings. Soil Science **3**: 547. 1917.

21. **Javillier, M.** La présence et le rôle du zinc chez les plantes. Thèse. Paris, 1908.

22. ——. Influence du zinc sur la consommation par l'*Aspergillus niger* de ses aliments hydrocarbonés, azotes et mineraux. Compt. Rend. **155**: 190. 1912.

23. **Jost, L.** Vorlesungen über Pflanzenphysiologie. 2te Aufl. Jena, 1908.

24. **Kahlenberg, L., and True, R. H.** On the toxic action of dissolved salts and their electrolytic dissociation. Bot. Gaz. **22**: 81. 1896.

25. **Knop, W.** Quantitative Untersuchungen über den Ernährungsprocess der Planze. Landw. Versuchsstat. Berlin **7**: 93. 1865.

26. **Kosinski, I.** Die Athmung bei Hungerzuständen und unter Einwirkung von mechanischen und chemischen Reizmitteln bei *A. niger.* Jahrb. Wiss. Bot. **37**: 137. 1902.

27. **Landolt-Börnstein.** Tabellen. 4 Aufl. **1**: 1189. Berlin, 1912.

28. **Latham, M. E.** Stimulation of Sterigmatocystis by chloroform. Bull. Torrey Club **32**: 337. 1905.

29. **Lepierre, C.** Sur la non-spécificité du zinc comme catalyseur biologique pour la culture de l'*Aspergillus niger.* Son remplacement par d'autres éléments. Compt. Rend. **156**: 258. 1913.

30. ——. Remplacement du zinc par le glucinum dans la culture de l'*Aspergillus niger.* Compt. Rend. **156**: 409. 1913.

31. ——. Remplacement du zinc par l'uranium dans la culture de l'*Aspergillus niger.* Compt. Rend. **156**: 1179. 1913.

32. ——. Remplacement du zinc par le cuivre dans la culture de l'*Aspergillus niger.* Compt. Rend. **156**: 1489. 1913.

33. **Lipman, C. B., and Gericke, W. F.** Experiments on the effects of constituents of solid smelter wastes on barley grown in pot cultures. Univ. Cal. Publ. Agr. Sci. **1**: 495. 1917.

34. —— ——. Copper and zinc as antagonistic agents to the "alkali" salts in soils. Amer. Journ. Bot. **5**: 151. 1918.

35. **Loeb, J.** Studies in general physiology II. Chicago, 1905.

36. **MacDougal, D. T., and Spoehr, H. A.** Growth and imbibition. Proc. Amer. Philos. Soc. **56**: 289. 1917.

37. **Mathews, A. P.** The nature of chemical and electrical stimulation II. The tension coefficient of salts and the precipitation of colloids by electrolytes. Amer. Journ. Physiol. **14**: 203. 1905.

38. **Mayer, A.** Untersuchungen über die alkoholische Gährung, den Stoffbedarf und den Stoffwechsel der Hefepflanze. Heidelberg, 1869.

39. ——. Lehrbuch der Agrikulturchemie. Heidelberg, 1871.

40. **Merck, E.** Chemical reagents, their purity and tests. 1914.

41. **Michaelis, L.** Die Wasserstoffionenkonzentration. Berlin, 1914.

42. ——**, and Marcora, F.** Die Säureproduktivität des *Bacterium coli.* Zeitschr. Immunitätsforschung **14**: 170. 1912.

43. **Migula, W.** Ueber den Einfluss stark verdünnter Säurelösungen auf Algenzellen. Dissert. Breslau, 1888.

44. **Mines, G. R.** The action of trivalent ions on living cells and on colloidal systems II. Simple and complex kations. Journ. Physiol. **42**: 309. 1911.
45. **Molisch, H.** Die Pflanze in ihren Beziehungen zum Eisen. Jena, 1892.
46. **v. Nägeli, C.** Die Ernährung der niederen Pilze durch Mineralstoffe. Sitzber. math.-phys. Classe Akad. Wiss. München 5 Juli, 1879.
47. **v. Nägeli, C.** Ernährung der niederen Pilze durch Kohlenstoff und Stickstoffverbindungen. München and Leipzig, 1882.
48. **Nikitinsky, J.** Ueber die Beeinflussung der Entwicklung einiger Schimmelpilze durch ihre Stoffwechselprodukte. Jahrb. Wiss. Bot. **40**: 1. 1904.
49. **Ono, N.** Ueber die Wachsthumbeschleunigung einiger Algen und Pilze durch chemische Reize. Journ. Imp. Coll. Sci. Tokyo, **13**: 142. 1900.
50. **Pantanelli, E.** Ueber Ionenaufnahme. Jahrb. Wiss. Bot. **56**: 689. 1915.
51. **Pasteur, L.** Mémoire sur la fermentation alcoölique. Ann. Chem. Phys. 3 sér. **58**: 323. 1860.
52. **Pfeffer, W.** Ueber Election organischer Nährstoffe. Jahrb. Wiss. Bot. **28**: 205. 1895.
53. —— Physiology of plants I. Oxford, 1897.
54. **Raulin, J.** Études chimiques sur la végétation des Mucédinées. Compt. Rend. **57**: 228. 1863.
55. ——. Études chimiques sur la végétation. Ann. Sci. Nat. V. Bot. **11**: 93. 1869.
56. **Reese, H.** Der Einfluss der gebrauchten Nahrlösung des Zinks und des Mangans auf das Wachstum von *Aspergillus niger*. Dissert. Kiel, 1912.
57. **Richards, H. M.** Die Beeinflussung des Wachstums einiger Pilze durch chemische Reize. Jahrb. Wiss. Bot. **30**: 665. 1897.
58. ——. The effect of chemical irritation on the economic coefficient of sugar. Bull. Torrey Club, **26**: 463. 1899.
59. **Robertson, T. B.** The physical chemistry of the proteins. New York, 1918.
60. **Russell, E. J.** Soil conditions and plant growth. London, 1917.
61. **Sauton, B.** Influence comparée du potassium, du rubidium et du caesium sur le développement et la sporulation de l'*Aspergillus niger*. Compt. Rend. **155**: 1181. 1912.
62. **Schulz, H.** Ueber Hefegifte. Pflügers Archiv **42**: 517. 1888.
63. **Smith, E. F.** Bacteria in relation to plant diseases. Vol. 2. Washington, 1911.
64. **Steinberg, R. A.** A study of some factors influencing the stimulative action of zinc sulphate on the growth of *Aspergillus niger*. I. The effect of the presence of zinc in the cultural flasks. Mem. Torrey Club, **17**: 287. 1918.
65. ——. A study of some factors influencing the stimulative action of zinc sulphate on the growth of *Aspergillus niger*. II. A comparison of two strains of the fungus. Bull Torrey Club, **46**: 1. 1919.
66. **Treadwell, F. P., and Hall, W. T.** Analytical chemistry. New York, 1916.
67. **Watterson, A.** The effect of chemical irritation on the respiration of fungi. Bull. Torrey Club, **31**: 291. 1904.
68. **Wehmer, C.** Entstehung und physiologische Bedeutung der Oxalsäure im Stoffwechsel einiger Pilze. Bot. Zeit. **49**: 233. 1891.
69. **Yasuda, A.** On the effect of alkaloids upon some moulds. Bot. Mag. Tokyo, **15**: 79. 1901.